TIME RESOLVED METABOLIC 13C MRS USING HYPERPOLARISED [1-13C]PYRUVATE IN A TRANSGENIC MAMMARY CANCER MODEL

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Introduction

The enzyme lactate dehydrogenase (LDH) is responsible for the conversion of pyruvate to lactate and the reversible reaction and hormonal up regulation of LDH expression has been observed in many breast cancer cell lines (1, 2). The tumours of the transgenic mouse breast cancer model MMTV-PymT (PymT) are characterized by a stepwise hormonal progression from benign into malignant morphology and resemble aspects of human breast cancer. The aim of this study was to assess the changes of LDH enzyme kinetics with time resolved hyperpolarized [1-^{13}C]pyruvate spectroscopy during tumour growth in a group of animals.

Our hypothesis was that information about the aggressiveness of the tumours could be assessed by the LDH rate constant, k_p (conversion of pyruvate to lactate) calculated from the [1-^{13}C]lactate and [1-^{13}C]pyruvate signals in the time resolved ^{13}C MR spectra.

Material and Method

Five PymT mice were scanned 2-3 times over a period of 4-6 weeks at various disease stages according to institution approved protocols. The animals were anesthetized using isoflurane. Respiration and temperature were monitored during the scanning session (SA Instruments, USA). Each scan session included a tail vein injection of 150 µL of 80 mM [1-^{13}C]pyruvate over 4-7 seconds. The [1-^{13}C]pyruvate sample was hyperpolarised in a HyperSense polariser (Oxford Instruments, UK) and dissolved in a buffer containing 80 mM TRIS, 100 mg/L EDTA, 50 mM NaCl, 80 mM NaOH (pH ~ 7.5-8, temperature ~ 35oC, isotonic osmolarity). The polarization of the samples was on average 25%. A 4.7T MR imaging and spectroscopy system (Varian Inc. USA) was used for all experiments. A ^{13}C surface coil for signal reception was placed over the tissue of interest and placed in a decoupled 13C/1H-tuned volume coil (RAPID Biomedical GmbH, Germany). A 3D gradient echo (GRE) sequence was used for tumour volume assessment (TR/TE/flip 3.64 ms/1.91 ms/40 deg, FOV 100x50x50mm^3, matrix 256x128x128, NEX 4). Dynamic ^{13}C spectra were acquired to measure metabolism as function of time after injection of [1-^{13}C]pyruvate. We acquired spectra from a 6-mm-thick slice with every 5th spectrum acquired from the entire sensitive volume of the surface coil for verification of signal level. The slice selective sequence used a flip angle of 10^o (TR 1 or 2 sec). Tumour volumes were manually delineated and calculated from the GRE sequence using ImageJ (3). The peak integrals of [1-^{13}C]pyruvate and [1-^{13}C]lactate were fitted in jMRUI (4) using the AMARES algorithm and fitted to a kinetic model based on Day et al (5) to obtain the rate constant k_p. The Day model was extended by adding a perfusion term to model the delivery of pyruvate to the tissue. The k_p values were plotted against the corresponding tumour volumes (figure 1). The resected specimens were evaluated for the presence of in situ and invasive carcinoma and for the presence of tumour necrosis**.** The pathologist was blinded to animal age.

Results and conclusion

We have used MR measurements of metabolic dynamics to extract information about the LDH rate constant, k_p. Our initial results show that we seem to be able to monitor the changes in LDH kinetics during tumour development. The trend of the curve in figure 1 shows an initial increase of the rate constant values reflecting increasing LDH activity with increasing tumour volume. The k_p values tend to decrease with larger tumour volume (> 300 mm^3). Our findings indicate that LDH activity correlate to the tumour volume and might be used as an indicator of tumour stage and for grading malignancy in mammary cancer.

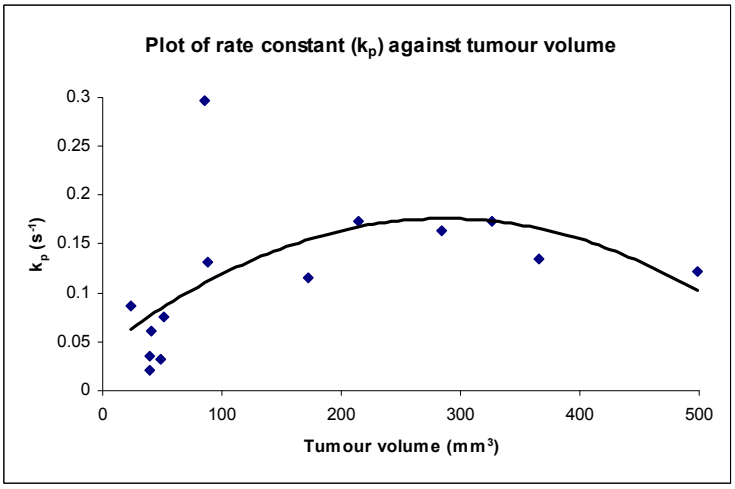

Figure 1: The rate constant plotted against corresponding tumour volumes. The volume of all tumours increased during the experimental period and dynamic ^{13}C spectra were acquired to measure metabolism as function of time after injection of hyperpolarised [1-^{13}C]pyruvate to extract k_p values.

References

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